The sensory morphology of insect pollinators
From structure to behaviour and ecology

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Abstract

Insect pollinators play an important role in balancing our ecosystems and maintaining plant and food diversity. Due to anthropogenic activities such as global warming, habitat loss and degradation, pesticides, and pathogens, many pollinator populations have been undergoing drastic declines in recent decades. Sudden changes in environmental conditions may lead to selection pressures to alter sensory systems, their structures and functions, and, consequently, behaviour. Despite the multitude of studies on insect pollinators and their population declines, these effects are often overlooked. To address this knowledge gap, I studied the morphology of sensory systems in butterflies and bumblebees – two pollinator groups found in temperate regions – from the aspects of development, behaviour, ecology and adaptation. In Chapter I, I used allometry to study how investment into sensory systems varies within and between different sexes of the butterfly Pieris napi. I measured the size and other parameters of sensory traits including eyes, antennae, proboscis, and wings. I showed that sensory system investment varies between sexes and only antennal length and wing size increase allometrically with body size. These findings suggest that not all sensory organs scale with body size and energetic investment between them can vary among sexes of the same species. In Chapter II, I explored the effect of a sub-optimal temperature on the development and morphology of sensory systems in P. napi, an ectothermic solitary insect. I exposed the pupae of P. napi to 23°C (optimal temperature) and 32°C (sub-optimal temperature) and measured their body and sensory organ size after emergence. I found that the mortality rate was higher at the suboptimal temperature and that the eclosion time decreased. Also, body and proboscis size decreased in both sexes, while antennal length decreased only in males. These results show that global warming can have negative consequences for the survival of butterflies and affect the size of their sensory systems potentially by accelerating the developmental process. In Chapter III, we studied the effect of heatwave-like temperatures on the sensory systems and behaviour of another insect pollinator, Bombus terrestris. Our results revealed that development in suboptimal temperatures had a negative impact on behavioural responses of bumblebee workers. Interestingly, the elevated temperature did not have a significant effect on the size of their antennae, eyes and forewings. These findings indicate that an elevated developmental temperature can impair important behavioural responses to sensory stimuli without causing any visible changes in sensory organ morphology. In Chapter IV, I explored how well the qualitative light micro habitat associated with a distribution range of insects, matches with carefully measured quantitative values. I used three butterfly species (P. napi, Pararge aegeria, Vanessa atalanta) that are associated with different light habitats and have different dispersal ranges. The results showed that only P. napi distribution was affected by light intensity. Eye and brain neuropil investment varied among the three species. P. napi had highest eye size investment while V. atalanta had highest optic neuropils investment. These findings suggest that visual and neural investments could only in part be associated with quantitative and/or qualitative light micro habitat and dispersal in these species.

Keywords: Insect pollinators, butterfly, bumblebee, sensory system, developmental temperature, light habitat.

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To Kavous Seyed-Emami, Sam Rjabi, Houman Jowkar, Niloufar Bayani, Morad Tahbaz, Taher Ghadirian, Amir Hosein Khaleghi, and Sepideh Kashani for their selfless efforts to save wildlife of Iran but treated with injustice and cruelty
The thesis is based on the following articles, which are referred to in the text by their Roman numerals:


Candidate contributions to thesis articles*

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* Contribution Explanation
Minor: contributed in some way, but contribution was limited.
Significant: provided a significant contribution to the work.
Substantial: took the lead role and performed the majority of the work.
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1. Introduction

1.1. Why studying insect pollinators

1.1.1. Small creatures, massive impact

Insect pollinators are small creatures, yet they play an important role in balancing ecosystems and maintaining flower and food diversity. When foraging, they spread the pollen grains they collect or that get stuck on their hairy body and proboscis (their tube-shaped tongue) among floral resources. Therefore, the viability and reproduction of most flowering plants on the planet rely on the foraging activity of these small creatures (Kearns et al. 1998). In addition to the pollination services they provide, insect pollinators are a vital part of the food web in most ecosystems, since they are valuable sources of energy for bigger animals such as birds (Black et al., 2011). As they are small and easy to rear in large numbers, insect pollinators are also common model systems for scientific studies. Despite being the focus of large research and conservation efforts, many pollinating insect groups are declining at an alarming rate: Global warming, habitat loss, habitat alteration and fragmentation, pesticides, insecticides, and pathogens are considered to be the major threats to their populations (Kremen et al., 2002, 2004; Gonzalez-Varo et al., 2013; Godfray et al., 2014; Goulson et al., 2015; Kerr et al., 2015; Soroye et al., 2020). Understanding which of these parameters, or their interactions, contribute most to the population declines we see is complicated, partly due to the high variation in morphology and life history among insect taxa (Gill et al., 2016). However, behavioural and ecological studies on foraging and pollinating activities, as well as mating and reproduction success, could help us map the effect of these threats and better understand their consequences for insect pollinator populations.

1.1.2. The importance of sensory systems for insect pollinators

Insects receive and process sensory information from their biotic and abiotic environment in order to forage, find and interact with conspecifics, reproduce and avoid predators. Sensory systems act as an interface that links the outside world of an insect to its central processing system (brain). However, the surrounding world of these small creatures is complex and cluttered with endless signals and noise. To overcome this, insects are equipped with specialized external sensory structures and an internal central nervous system that enhance and amplify relevant signals and filter noise and irrelevant cues through matched sensory filters (Wehner, 1987; Warrant, 2016). Because sensory systems come with energetic costs, insects tend to develop functional,
physiological and anatomical specialisations that are optimised for the sensory
cues that are available in their habitat. Rapid changes in environmental condi-
tions may lead to selection pressures on sensory systems, hence alterations in
their structure and function. Depending on the type of change and the devel-
opmental plasticity or an organ, these responses may be adaptive or maladap-
tive (Niven & Laughlin, 2008). Several studies have provided examples of
morphological changes in insect sensory organs in response to environmental
changes. For example, Gérard et al. (2023) found that absolute antennal length
and relative proboscis length decrease if bumblebees (Bombus terrestris)are
exposed to an elevated temperature (33°C) during development. Exposure to
elevated temperature has also been shown to affect the function and sensitivity
of olfactory receptors in Drosophila (Riveron et al., 2009; Martin et al., 2011).
These changes are likely to impact insect pollinator behaviour and are poten-
tially an underestimated factor in their population declines (Gérard et al.,
2023). Nonetheless, we lack answers to the following questions: 1. How, and
to what extent, are the sensory systems of insect pollinators sensitive or resil-
ient to environmental changes? 2. How are these changes reflected in the be-
haviour and survival of insect pollinators? To address these questions, the first
step is to understand how sensory systems are structured and adapted, and how
they function.

1.1.3. Sensory systems in insects

In general, the external sensory systems in insects are made of anatomical
structures that provide a surface for sensory receptor cells (Hill et al. 2004)
that are located in cuticular sensory hairs (sensilla) and cover most insect body
parts, or, in the case of compound eyes, the photoreceptor cells in ommatidia.
The function of sensory receptor cells is to receive stimuli from the surround-
ing environment and convert it to electrical signals that are transferred to the
brain where the information is processed (Hill et al. 2004). Like most insects,
insect pollinators are mainly equipped with visual, olfactory and gustatory
systems that are also sensitive to mechanical, hygro and thermal stimuli (Su-
wannapong & Benbow, 2011).

Visual system

For many diurnal insect pollinators, vision is the most important sensory
system. Sampling and collecting visual signals from the sky, landmarks and
food sources allows for visually guided behaviours such as navigation, orient-
ation learning and memory for dispersal (de Vries et al., 2017). Vision also
plays an important role in finding mates and specialized visual adaptations can
be found in males of several insect species (Land, 1989, 1990).
A pair of compound eyes comprise the main external visual system in insects.
There are three different types of compound eyes – apposition, neural super-
position and refracting superposition (Greiner, 2006) – that each reflect the
visual requirements and light habitat of the insect. For example, the structure
of the apposition eye functions best for insects from bright habitat (Frederiksen & Warrant, 2008). Apposition eyes consist of isolated ommatidia made up of a corneal lens/facet, a crystalline cone (dioptic apparatus) that projects the light incidents into rhabdom, a photosensitive structure made out of retinula cells (Warrant et al., 2003; Von der Emde & Wrrant, 2015). Retinula cells transform absorbed light into a neural signal, that is subsequently transmitted to the lamina in the optic lobe of the brain (Ribi, 1974; Meinertzhagen, 1976). The lamina is the most peripheral visual processing area in the brain and is responsible for generating contrast, in addition to processing other types of visual information. The other specialized optic neuropils are the medulla, the lobula and the lobula plate. The medulla receives visual input from the retina and the lamina and processes motion, colour, and polarization signals while the lobula and the lobula plate (notably absent in bees) are mostly tuned for analyzing optic flow and detecting motion (Cronin et al., 2014). The optic neuropils are linked to higher order sensory processing systems in the brain including the central complex, the anterior optic tubercle and the mushroom bodies. In these neuropils visual information is further processed and integrated with other sensory information (Homeberg, 1987, 2008; Mota et al., 2013; Kinoshita et al., 2015).

Visual adaptations of perceiving light

Even among diurnal insects, one can find different morphological adaptations in the compound eyes and the visual processing areas of the brain to achieve optimal sensitivity and resolution (Scales & Butler, 2016; Stöckl et al., 2016; Couto et al., 2020; Bartholomée et al., 2023). Sensitivity and resolution in compound eyes can be increased by increasing the size of the eye. From this point, there are two options. Increasing facet size which leads to improve visual quality by increasing the size of facets or receptors to increase the rate of photon capture and improves light sensitivity but the resolution will be compromised. Alternatively, increasing the numbers of facets which increases resolution, but reduces light intensity since they sample smaller angles of space (Kirschfeld, 1976; Land, 1997). Therefore, there is always a trade-off between sensitivity and resolution in compound eyes. Subsequently, the visual features of the compound eyes in insects are adapted to their specific light habitats (Warrant, 2001). Distinct adaptations are even common between conspecifics and among sexes due to their behavioural roles (Rutowski, 2000; Spaethe & Chittka, 2003). For example, sexual dimorphism of eye size is found in most butterfly species (Rutowski, 2000; Ziemia & Rutowski, 2000). Interestingly, even within the eyes, visual features are locally adapted based on the insect’s ecological and behavioural needs. This means that, along the eye topology, different regions have different facet diameters to optimize vision to the needs of the individual while minimizing energy investment. The primary (lamina, medulla, lobula and lobula plate) and higher order (mush-
room bodies, the anterior optic tubercle and the central complex) visual processing areas in the brain can also improve visual sensitivity. For example, morphological investment in the size of the optic neuropils have been associated with the light intensity of the insects’ habitats (Bouchetbi and Arganda, 2020). Species from bright habitats have found to invest in these neuropils more than species from dark habitat and nocturnal species (Stöckl et al., 2016, Kinoshita et al., 2015). In addition, investment in the areas of the central brain that are responsible for compass orientation has been found in migratory species of butterflies, moth and desert locust (el Jundi et al., 2011, Heinze and Reppert, 2011)

**Olfactory system**

The olfactory system is another important sensory system for insect pollinators in many purposes. Conspecific communication via chemical odor detection is important for eusocial insects in order to distinguish colony members and detect intruders but also to help them find flowers that are scented by their conspecifics (Pearce et al., 2017). Insects also use olfaction for sexual purposes such as pheromone detection when searching for mates. Another important role of the olfactory system is detecting host plants for laying eggs (Ikeura et al., 2010) and for locating food resources at close proximity. Olfactory information is often combined with other sensory cues (Andersson & Dobson, 2003, Heinz, 2008), such as vision, to increase accuracy.

The external structure of the olfactory system in insects is a pair of often elongated antennae that contain olfactory sensilla (sensory receptor hairs). Olfactory sensillae contain many pores in their walls that facilitate penetration of odour molecules. These sensillae have different morphologies and sizes, they can be long-hair shaped, short and groove shaped or hidden in pits with small apertures that let air molecules penetrate into the sensillum lymph (Steinbrecht, 2007). In the lymph, the odor molecules are detected by specialised receptors on the end of neural dendrites. Olfactory information is then received by a pair of antennal lobes that are the first region of olfactory processing in the insect brain. Each antennal lobe contains several olfactory glomeruli that cover the outside of the lobe and link receptor axons via synaptic contacts. The intraglomerular axon then projects the olfactory information into the mushroom body calyx via the antennal-cerebral tract (Hansson & Anton, 2000) for further processing. The olfactory information processing in mushroom bodies has been related to formation of mid- and long-term olfactory memory in insects (Menzel, 2001; Davis, 2011). In addition to the antennae, some insects, such as butterflies, have maxillary or labial palps are also sensitive to olfactory cues. In this thesis, however, I mainly focus on the antenna. In addition to olfactory sensillae, insect antennae are also covered with other types of sensillae that are sensitive to temperature, humidity and mechanical stimuli (Schneider, 1964; Hansson & Stensmyr, 2011). Antennae have also
been suggested to aid in long distance migration in monarch butterflies (Danaus plexippus) by detecting time-compensated sun compass and magnetic cues (Merlin et al., 2009; Guerra et al., 2012; Guerra et al., 2014).

**Adaptations to perceive olfactory cues**

The morphological shape of the antenna as well as the types and numbers of sensilla are diverse among insect taxa but even among different casts of eusocial insects and different sexes of the same species. For example, the flagellum is geniculate-elbowed shape in bees but club-shaped in butterflies though it is not completely clear how these differences evolved. Increasing the size of the antenna provides a larger surface for accommodating more sensilla and thus improves sensitivity. Sociality seems to be one of the factors that shapes such adaptations. Comparative phylogenetic analysis suggests that eusocial insects have morphologically evolved antennae that are more sensitive than solitary insects (Kocher & Paxton, 2014). Even between bumblebee workers, those with larger antennae have more olfactory sensillae and thus a higher sensitivity to olfactory odors (Spaethe et al., 2007). Foraging behaviour is another possible driver of increasing olfactory sensitivity in insect pollinators. Using olfaction for foraging can be particularly challenging for generalist species of pollinators as they have to discriminate between a multitude of odour signals among flowers. Therefore, they should have developed antennae with higher sensitivity which is potentially possible by expanding the surface of the antennae to increase sensilla numbers (Chapman, 1982). Olfactory adaptations can also occur on the neural level and in the central nervous system. For example, olfactory receptor neurons can be specialized to detect certain odour cues, e.g. one specific component of a sex pheromone (Hansson, 1995). Also, sexual dimorphism in the numbers of olfactory glomeruli has been reported in butterflies and moths (Rospars & Hildebrand, 1992; Huetteroth & Schachtner, 2005; el Jundi et al., 2009, Montgomery & Ott, 2015).

**Gustatory system**

Gustation is the sensory modality that facilitates feeding and this way aids pollination activity. The task of the gustatory system is more specialized, and, regardless of its importance, less is known about it compared to the other two senses discussed above (Newland et al., 2000). Like the olfactory system, the gustatory system detects chemical stimuli in the environment, mainly from food but also other chemical signals relevant to the insect. The gustatory receptors in insect pollinators are usually accumulated in the elongated tube-shaped mouthpart called the proboscis that evolved to facilitate feeding on fluids such as nectar. The proboscis contains several chemoreceptors and mechanoreceptors shaped as sensilla, similar to the ones found on antennae. The primary area of gustatory information processing that links the proboscis to the brain is a complex fused ganglion called the subesophageal ganglion (Vajna et al., 2021). Chemoreceptors sensitive to gustatory cues can also be
found on other insect body parts such as tarsi, antennae, ovipositor and wings (de Boer & Hanson 1987, Ramaswamy1988, Städler & Retoschöni 1991; Chapman 2003). Among these, in this thesis I mainly studied the proboscis.

**Adaptation to perceive gustatory cues**

While there are several studies on the morphology and types of sensilla in the proboscis (Hu et al., 2021; Molleman et al., 2005), relatively little is known about the possible adaptations of this sensory system in insects. Size and variations in proboscis structure are perhaps the most prominent gustatory adaptation in insect pollinators that can be correlated to the shape and structure of their food and feeding efficiency. In social insect pollinators, such as bees, proboscis length has been linked to selection of flowers (Goulson et al., 2008; Peat et al., 2005), pollination efficiency (Stout et al., 2000; Shimizu et al., 2014), community assembly (Harmon-Threatt & Ackerly, 2013), and plant pollinator interactions (Stang et al., 2006). In butterflies, it has been suggested that the narrow and flexible shape of the proboscis is suited for feeding from slender corolla (Barth, 1991). The types of sensilla in the proboscis and its cuticular structure have also been suggested to relate the type of fluid on which Lepidopterans feed, (Monaenkova et al., 2012; Lenehert et al., 2013; Hu et al., 2021), likely related to a particular sensitivity to chemical odours and fluid viscosity. One of the most interesting feeding adaptations, however, has evolved in some families of neotropical butterflies, Heliconius and Laparus, that feed on pollen with their proboscis. Surprisingly, in these species, the anatomical structure of the proboscis and most sensillae are not very different from what is found among other Nymphalid butterflies. However, compared to non-pollen feeding butterfly species, these groups have a more elongated proboscides with longer mechano-receptive bristles that have been suggested to help with the collection of pollen (Krenn & Penz, 1998). Lastly, the size of the proboscis likely has an effect on the sensitivity of this sensory organ since increasing the surface allows for accommodating more gustatory receptors.

1.2. Why Butterflies and Bumblebees

Bumblebees and butterflies are two pollinating insect groups that have successfully populated the challenging climates of temperate regions. They both are holometabolous, meaning that they go through complete metamorphosis during their life cycle (egg, larvae, pupae, imago/adult). They often have overlapping ecological niches, and can forage and feed from the same floral patches. They both use flight for increasing foraging efficiency and finding mates, for example. They can be found in dark forests and bright open lands and they use chemical signals for mating and conspecific communications. However, there are also differences in many other ecological, behavioural, evolutionary and life history aspects that distinguish them from one another.
For example, they have different physiological responses to changes in ambient temperature. While all butterflies are ectothermic, their body temperature is dependent on ambient temperature, bumblebees are facultative endotherms which means that they can regulate their body temperature to some extent by increasing the temperature of their thorax. Lifestyle is another factor that varies between these two insect groups, with bumblebees being eusocial, while butterflies are mainly solitary. Lastly, while the external structures and the main functions of the sensory systems in bumblebees and butterflies have similarities, they can have distinguishable morphological, structural, functional and even physiological differences. These differences can be found from the external structures to the receptor and neural levels. One of the distinguishable differences is between the visual adaptations in these two species. In bumblebees, apart from the two compound eyes, they have three camera-type eyes on the dorsal area of their head, called ocelli, which are not present in butterflies. The similarities and differences between these two groups, make them interesting models for exploring how the sensory systems of insect pollinators might be shaped by their ecological niches. In addition, how insect pollinators in general might respond to sudden changes in their habitats.

1.2.1. Introduction to the study species

Bombus terrestris

This species of bumblebees has several subspecies and mainly occupies temperature regions, although they tend to be habitat generalists, and occupy forests, semi-natural grassland and urban areas (Kämper et al., 2016). They are central-place foragers that can fly up to several kilometers from their nest (Osborne et al, 2008) and can extract nectar and pollen from many types of floral species. They often build their nest underground where the colony grows. These species of bumblebees are eusocial with the queen responsible for reproduction and establishing the colony, and workers that perform different tasks, such as foraging or taking care of the brood. Their physiological and social strategies for coping with a broad range of climatic conditions, as well as their generalist foraging strategies means that sensory systems of this species should be adapted in a way to be capable of detecting multitudes of sensory stimuli.

Pieris napi

This species of butterfly is common in temperate zones and has a wide global distribution, from Europe to Asia and North America, with a high occupation in northern latitudes. They are ecological generalists, meaning that they can be found in a wide range of habitats, including natural and semi-natural grasslands, arable lands, woodlands and urban areas. Similar to B. terrestris, they also forage on several types of flowers. Populations are mostly localised but they also disperse sometimes over longer distances. Males
emerge earlier and visually search for female (Forsberg & Wiklund, 1988) while females emerge later and search for host plants for oviposition (Wiklund & Forsberg, 1991). This species from the Pieridae family has been recently found to be cold tolerant, which can potentially make them vulnerable to increasing global temperatures. However, they have better performance and population viability during colder periods (von schmalensee et al., 2023). Therefore, this species is a good candidate for the purpose of our research, as it is a generalist species like *B. terrestris*.

*Pararge aegeria*

Unlike *B. terrestris* and *P. napi*, this species is mostly found in woodlands, boreal forests and forest borders. Adults mainly feed from maple honeydew but can also feed from floral nectar. Males find females by either patrolling (searching for females while in flight) or perching (perching in sunny spots in the forest waiting for females to fly past), both of which are visually driven behaviours. Females of this species can fly up to the tree canopies in the forest or fly slowly over the grasses to search for host plants and to lay eggs on shaded plants in summer (Eeles, 2019). This species is therefore interesting to study for its adaptation to darker and more visually complex habitats.

*Vanessa Atalanta*

This species can be found in wide range of habitats, such as semi natural grasslands, forest borders and gardens, although it mostly avoids darker habitats such as forest. Adults feed from multiple sources of sugar, which they gain from the nectar of a wide ranges of flowers, tree sap or rotten fruit. Like with *Pararge aegeria*, males of this species are also patrollers or perchers (Eeles, 2019). Among all the model species in my thesis, this species has the longest dispersal and is considered as migratory. They become sexually mature and mate shortly after migration (Brattström, 2007). The long-distance dispersal of this species may result in them developing sensory systems that are specialised for migration and for passing through multiple types of ecosystem. However, compared to migratory monarch butterflies (*Danaus plexippus*), little is known about the sensory adaptations of this species.

2. Aims and methods

2.1. Objectives

Changes in the habitats of insect pollinators, either due to habitat alterations or climatic changes, can modify their environment in a way that they might not be adapted to. These changes can happen over a particular stage of development or through the entire developmental cycle, which may lead to changes
in the morphology and function of sensory systems, and consequently behaviour. In my PhD thesis, I aimed to improve the knowledge about the understudied effect of environmental changes on insect pollinator sensory systems and how this affects their ecology, behaviour and pollination services. I used and developed several morphological and behavioural techniques to explore the morphological adaptations of insect pollinators in different stages of development. In this section, the methods, findings and their interpretations, from my four thesis chapters are summarised.

2.2. Methods

2.2.1. Morphological techniques and tools

Morphological studies, in which the shape, size and structure or organs is analysed, are useful for understanding the possible adaptations of sensory systems in insect pollinators. Morphological analyses of insect sensory systems help us to detect any modifications in size and structures at different resolutions, from large to fine scales. Imaging and microscopy are the two prominent tools in morphological studies that I also applied in all of my projects. One of the imaging techniques that has advanced morphological studies in recent years is X-ray micro computed tomography (micro-CT). This technique has developed the possibility to acquire high resolution (micron to sub-micron) 3D image stacks that can be reconstructed to generate a digital 3D model of a specimen (Baird & Taylor, 2017, Figure 1). I used this technique in chapters I, II, III & IV, to measure morphological features of the compound eye such as the surface area, ommatidia diameter and density, interommatidial angle (the angle between two adjacent ommatidia) and eye parameter (the product of facet diameter and IO angle). I also collected several brain samples with this method to analyse 3D brain structure and neuropil volume of the three studied species in Chapter IV. This method has several benefits compared to traditional histological techniques for studying eye morphology. For example, this method is non-destructive and can be used several times at different resolutions and objectives to detect the region of interest that may not otherwise be visible externally (Baird & Taylor, 2017). It is also faster and provides the possibility of collecting larger sample sizes that are more statistically reliable. To segment and reconstruct the acquired 3D images from micro-CT, I used 3D image processing software including Amira (Thermo Fisher Scientific) and an open source visualization program, 3D Slicer image computing platform.

To measure the length of the antennae and proboscis in chapters I, II & III, as well as of the wing (chapter III), manual photography was used, and their length was measured in image J. To measure forewing area in chapter I and thorax width in chapters I & II, the images were analysed using an in-house
automated program in python. Photographed forewing area and shape was measured using geometric morphometric analysis in chapter II.

**Figure 1.** In X-ray micro computed tomography method, a rotating sample is illuminated by using x-ray radiation. On a detector, the interactions between the x-rays and the sample are imaged. A computer algorithm is utilized to combine images taken from various angles, thus reconstructing a digital image stack that portrays the three-dimensional structure of the sample. This data volume can then be analyzed for segmenting and quantifying the sample’s surface or internal features (Adopted from Baird & Taylor, 2017).

**Methods in chapters**

In chapter I, to explore how the energetic investments are distributed among sensory systems and whether there are any sex-specific differences between sensory investments, I performed a comprehensive allometric study on males and females of butterfly *Pieris napi*. Allometry, which is the study of understanding how organ size scales with body size, is a useful method to understand investment among traits in relation to the growth of body size. I measured thorax width (as a proxy of body size) as well as eye surface area, antennal length, proboscis length and forewing area and used allometric relationship $\log_{10}(Y) = \log_{10}(b) + \alpha \log_{10}(x)$ to see whether any of these traits scale with body size. In this equation, $Y$ corresponds to trait size, $x$ corresponds to body size, $\alpha$ is the allometric coefficient i.e. the slope and $b$ is the intercept (scaling shift). If the trait and body scale at the same rate, where $\alpha = 1$, the relationship is isometric. If the trait increases at a higher rate than body
size, $\alpha > 1$, the relationship is hyperallometric. If the trait increases at a lower rate than body size, $\alpha < 1$, the relationship is hypoallometric.

Specimens used in this study were the F2 and F4 lab reared generations originated from a wild population of *P. napi* collected in Ransvik, Southern Sweden. The eggs were placed in a rearing room at 23°C with 22:2 h L:D lighting regime and 100% humidity until hatching. Newly hatched larvae were kept at the same condition as eggs until pupation, while F2 were fed with Alliaria peiolata and F4 with *Brassica napus* and *Armoracia rusticana*. Newly pupated specimens were collected every day, sexed and placed in a climate chamber at 23 °C, 22:2 h L:D lighting regime and average humidity: 31% until emergence. Adult butterflies with full development were then dissected after anesthetized with ethyl acetate for traits measurements.

In chapter II, the effect of sub-optimal temperature on development, mortality and morphology of body and sensory organs was investigated on the ectothermic solitary butterfly *P. napi*. I exposed the pupae of *P. napi* to 23°C (optimal temperature) and 32°C (sub-optimal temperature) and measured their body and sensory organ size after emergence (Figure 2). The 23°C was chosen as optimal temperature since it lies within the range of summer temperature that pupae of this species develop directly (Larsdotter-Mellström et al., 2015) while 32°C was chosen as sub-optimal because this temperature is the upper limit of pupal survival of *P. napi* (von Schmalensee et al., 2023). In addition, following the increasing temperature in summer in Sweden, this temperature seems to be more frequently experienced during the development time of this species. The specimens used in this experiment were from the same F2 generations as used in chapter I and were reared under the same conditions until pupal stage. At the pupal stage, specimens were randomly assigned to one of two temperature treatments (23°C: optimal temperature and 32°C: sub-optimal temperature) until emergence. Mortality rate was accounted for after the last viable individuals emerged (4 weeks from pupation). Pupal development time was calculated for each temperature treatment based on the duration of chrysalis formation until adult eclosion. The collection of traits samples was performed following the methods described in chapter I. The absolute size of the traits was accounted for testing the effect of temperature on the size of these traits.
Figure 2. In this image, an adult is depicted alongside body and organ morphological measurements. These measurements include thorax width (indicated by an orange arrow within a rectangle bounding box), forewing measurements utilizing 11 landmarks (depicted as red dots with cyan numbers), proboscis length (highlighted by a red arrow), and antennal stalk length (illustrated with a light blue arrow). Inset: A 3D rendering of a butterfly head showcases the segmented left eye area, marked by gold lines (Adopted from Moradinour et al., 2023)

In Chapter III, the effect of sub-optimal developmental temperature was studied on another species of insect pollinators, *Bumbus terrestris*, a facultative endothermic insect with eusocial lifestyle. We asked whether exposure to sub-optimal temperatures over the mid-late stages of development can affect body and sensory system morphology and/or the function and thus the behavioural response of the bumblebee workers. Eight colonies of commercial bumblebees consisting of 1 queen and between 19-40 workers were used for this experiment – 4 colonies were kept in a climate chamber at 26°C and the other 4 at 33°C in dark conditions upon arrival to the lab. Temperature as high as 33°C, has been found to increase worker thermoregulatory behaviour to reduce the temperature of the colony, which can be energetically costly (Vogt, 1986). Studies have shown that temperatures higher than 30°C can negatively impact the numbers of workers and colony life span (Nasir et al., 2019). All the bees from both treatments had ad libitum access to sugar water and to organic pollen that was replenished every 2-3 days. After 7 days exposure to the temperature treatment, all individuals that were fully developed or were in nearly full development stage were marked. Over the course of 11 to 20 days of exposure, individuals without prior markings were tagged with individual number and colony colour.
Each individual caught from the colony was placed in the experimental setup in dark condition for 2 min for acclimation. After that, she was exposed to seven stimuli sequentially with a 2 min break in between, at an ambient temperature 23°C. The behavioural response of the bee to each stimulus was then recorded by a camera under the red light, and was classified as ‘expected’ or ‘unexpected’ by an experimenter blind to the bees’ treatment group. The behavioural setup used and tested sensory stimuli is described in Figur 3. Antennal length, eye area and forewing size was measured according to the methods used in chapters I and II. The binary response to each sensory stimulus and the absolute size of each organ was analysed to investigate the effect of experimental temperature.

**Figure 3.** An illustration of a behavioural arena experimental set-up. The setup was employed to investigate the impact of rearing temperature on the behavioural responses of bumblebees towards various sensory stimuli. Within this setup, bees were positioned at the center of the arena and exposed to different stimuli, which included a light stimulus from above (either UV or white light), an olfactory or mechanosensory stimulus delivered from below using a syringe (honey water odor or an air puff), and a liquid drop placed on the arena floor (either white sugar water or quinine). To monitor these responses, a camera was used to record the setup from an overhead perspective (Adopted from Perl et al., 2022).
In Chapter IV, the aim was to understand how well the qualitative light habitat associated with the distribution range of insects matches with detailed quantitative measurements of light microhabitat as well as visual and neural systems’ morphology. To address these questions, I studied three species of butterflies (Pararge aegeria, Pieris napi and Vanessa atalanta) from the same geographical region but with different dispersal ranges. I performed a series of transect walks in the areas that have a high abundance of these species, and sampled the light, temperature, condition (sun/shade) and habitat type (forest/forest edge/semi-natural grassland) in the spots where I observed individuals. On the last day of transect data collection, I randomly collected a few individuals from the study sites for morphological study of the eye and brain. The abundance of each species across the range of light intensities present in their habitat, as well as their frequency distribution in each condition and habitat type were tested. Allometric analysis was used to explore the visual and neural morphological adaptations in the three species.

3. Major findings and interpretations

3.1.1. What morphological study tells us about sensory investment in a generalist butterfly?

In chapter I, the analysis from allometric relationships revealed that, among all sensory systems measured in this study, only the antenna was scaled allometrically with body size in P.napi and the slope was hypoallometric. This is an indication of higher investment in relative size of the antenna in larger individuals compared with their smaller conspecifics. This relationship was significantly different between males and females with a greater antennal investment seen in females. Olfactory sensitivity can be an important sensory modality for females to detect male sex pheromone as well as chemical substances of the host plants (Schäpers et al., 2015). At the sensory receptor level, I did not find any significant relationship between the club size (the area that olfactory sensilla are mainly located) with the density of olfactory sensilla, suggesting that larger clubs can accommodate a larger number of olfactory receptors and are therefore likely to be more sensitive to olfactory cues.

I also found a positive hypoallometric relationship between forewing area and thorax width, with almost the same slope for both sexes. Scaling the thorax width with wing size is important for supporting flight, as larger individuals would need greater thoracic muscle mass to move their larger wings (Berwaerts et al., 2002; Le Roy et al., 2019). No allometric relationship was found between eye and proboscis with body size. Regardless of relative size, the absolute size of the eye and antennae in males was bigger than in females, suggesting a sexual dimorphism of these sensory traits.
3.1.2. How elevated developmental temperatures affect sensory systems morphology (in butterflies and bumblebees) and behaviour (bumbleees)?

Exposure to sub-optimal temperature during the last stages of development had different effects on the morphology of the body and sensory systems in the two species of insect pollinators, *P. napi* and *B. terrestris*. In chapter II, I found that *P. napi* pupae that were exposed to 32°C had higher mortality, faster development, smaller body size, shorter proboscides (in males and females) and shorter antennae (only in males) than pupae reared at 23°C (Figure 2). However, I found that eye size, as well as wing size and shape, were not affected by developmental temperature. These findings suggest that there might be a difference in thermal sensitivity between these organs that might be an adaptive or maladaptive mechanism to cope with seasonal variations. Exposure to sub-optimal temperature during the later stages of pupal development did not affect the size of any of the bumblebee workers organs and body mass during the mid-late stages of development, reported in chapter III. It is intriguing that organs were unaffected by sub-optimal temperature in our study, but one possibility might be due to the exposure duration in our study compared to other studies where morphological changes has been detected during constant elevated temperature during development (Gerard et al., 2018; Gérard et al., 2023). However, the exposure duration to sub-optimal developmental temperature was sufficient to affect the sensory responses of bees to the presented stimuli. Bees that were exposed to 33°C had 8% higher unexpected responses to the presented stimuli compared to bees from 23°C suggesting negative consequences of sub-optimal temperature to bumblebee behaviour (Figure 5). Considering no morphological effect on the body and organs of bumblebees from high temperature were found in our study, it is likely that behavioural impairment we observed may have a neurological rather than morphological basis. Overall, the findings of chapters II and III indicate that increasing global temperatures will have negative consequences for the development and function of sensory systems in insect pollinators. These effects can appear at different stages (heat-wave conditions) or throughout the entire development (constant elevated temperature) and can cause changes or impairment in sensory systems at different morphological, functional and neural levels depending on the exposure time and temperature.
Figure 4. Figures illustrating the effect of developmental temperature and sex on the size of body parts and sensory organs in *P. napi*. A) Pupal development (*n* = 62), B) thorax width (*n* = 47), C) proboscis length (*n* = 42), D) antennal length (*n* = 48), in females (purple) and males (green). The significant differences have shown with different letters. (Adopted from Moradinour et al., 2023)
Figure 5. The study examines how developmental heatwaves influence the proportion of expected and unexpected behavioral responses in bees that have been exposed to (A) air puff, (B) brown sugar water, (C) honey odour, (D) quinine, (E) UV light, (F) white light (G) white sugar water. (H) For all presented stimuli, the total proportion of expected and unexpected behavioral responses. The Sample size in this test is reported 59. (Adopted from Perl et al., 2022).

3.1.3. What does the quantitative assessment of the light habitat tell us about distribution and visual adaptations of butterflies?

In chapter VI, I found that quantitative light microhabitat only affected the abundance of *P. napi* which was at the highest peak of light intensity recorded in this study (10-12 lx.log), but not *P. aegeria* and *V. atalanta*. However, all the three species were found across a wide range of light intensity and had a peak that overlapped with one another in the bright side of the light spectrum. When the abundance of each species was assessed based on the habitat type (forest vs. open) I found that *P. aegeria* has the highest abundance in the forest compared to the two other species, while the majority of the *P. napi* and *V. atalanta* found in open habitats. I also found that *P. napi* had the largest eye size, *V. atalanta* had the largest optic lobes and *P. aegeria* had the largest anterior optic tubercle. Overall, I found a shift scale among all the brain neuropils and eye size between the three species. These findings suggest that light quantitative light micro habitat does not entirely associate with butterfly distribution. Visual neural investment had interspecific variations that only in part could be explained by light habitat associations and dispersal range.
Figure 6. The inter-specific distribution along the light gradients. The abundance for each species is illustrated accordingly: A) *P.aegeria*: Demonstrates a constant level with no discernible trend. B) *Pi.napi*: Exhibits a unimodal skewed pattern. C) *V.atalanta*: Displays a unimodal symmetrical distribution.

4. Concluding remarks, limitations and suggestions

In this thesis, I highlighted the importance of sensory traits morphology in insect pollinators to better understand their susceptibility to environmental modifications. The take home messages of the four chapters of my thesis are:

1. Variations in the morphology of sensory systems can provide some knowledge about the ecological and behavioural needs of an insect. Allometric analyses are a useful tool to understand how energetic investment is distributed among the different functional traits of an insect. However, there are challenges and limitations that need to be taken into consideration when studying and interpreting sensory system investment, particularly in butterflies. Variation in techniques and the choice of body organ or body mass as a proxy of body size as well as dissection procedures in morphological studies can make the interpretations of the results more challenging. Butterfly organs are delicate and sensitive to dry conditions and mechanical force, therefore collecting intact samples is often difficult and time consuming. I tried to develop methods to facilitate and speed up the process of sample collections in this thesis, but there is room for improvements that future studies with the help of technology and perhaps AI could potentially solve.

2. Increasing global temperatures impact the development of insect pollinators but also the morphology and function of sensory systems. The duration of exposure, fluctuating or constant temperature, stage of the development, species, sex, life history, physiology, adaptation and
seasonal plasticity, as well as the co-stressors that interact with temperatures are important parameters that need to be considered and interpreted with caution.

3. Light habitats of the flying insects does not always reflect on their visual and neural morphological investments and factors such as dispersal range, phylogeny, life style, sex and habitat characteristics such as canopy cover and may affect these investments. Visual and neural adaptations can occur at the finer characteristics of visual system and its respective visual processing areas in the brain, therefore they should be considered along the overall structures of these organs.
5. References


6. Sammanfattning

spridningsområden. Resultaten visade att endast fördelningen av *P. napi* påverkades av ljusintensiteten. Investeringen i ögon och hjärnnervvävnad varierade mellan de tre arterna, men denna variation hade ingen tydlig relation till deras kvalitativa eller kvantitativa ljusmiljö. Därför kunde jag visa att noggrann bedömning av ljusmiljöer är avgörande för att förstå och tolka visuella och neurala anpassningar hos insekter.
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Zan, Zendegi, Azadi...